Supplementary Figures

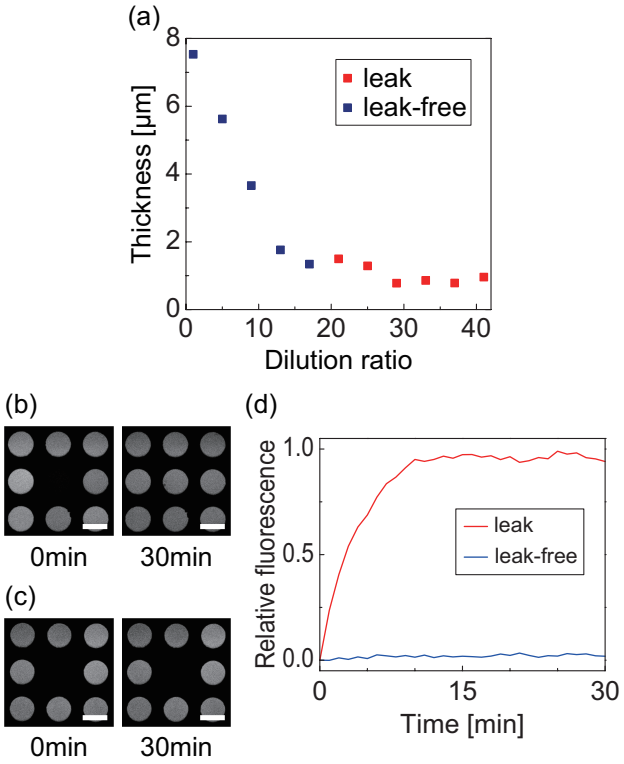

Figure S1: The evaluation of the hermetic sealing of the lid after enclosing the solution. (a) The thickness of the PDMS coated on top of the quartz was varied by diluting the PDMS with hexane. The thickness was measured with a laser scanning microscope (VK-9700/9710; Keyence). FRAP experiments with the chamber coated with PDMS of (b) 0.8 and (c) 1.7 μm heights are shown. 2 μM GFP was first encapsulated in the glass microchamber and one of the chambers was photobleached. The images show the micrograph of the chambers immediately after photobleaching and after 30 min. The scale bars are 30 μm. The time course of the relative fluorescence intensity of the bleached chamber is shown in (d), where 1 and 0 are the fluorescence intensities before and after photobleaching, respectively. Similar results were obtained with fluorescein (not shown).

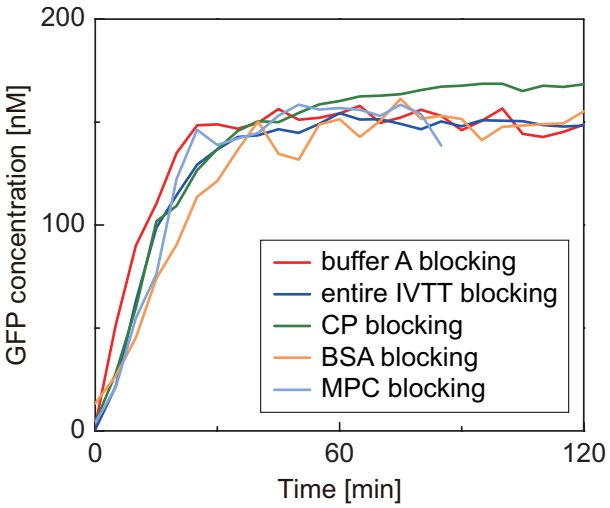

Figure S2: The GFP synthesis with glass microchambers after the blocking treatment. The blocking reagents used were 10% bovine serum albumin (BSA), 0.5% 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer, the entire IVTT solution, 50 mM creatine phosphate, or buffer A. We did not observe any effect on the time course of the GFP synthesis with any of the reagents used.

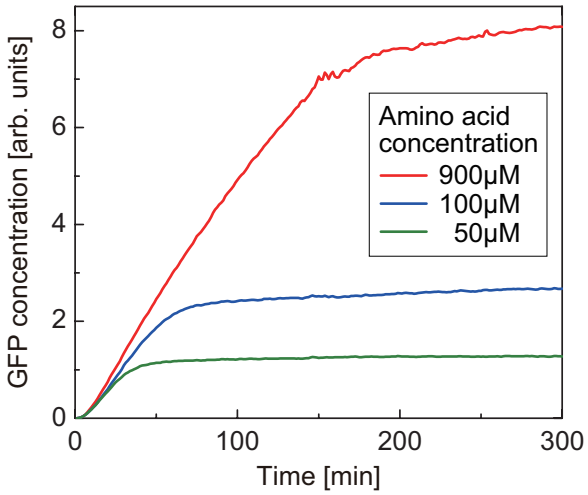

Figure S3: The effects of reducing the amino acid concentration in the IVTT used in this study. With 50 or 100 μM amino acids (a mixture of 20 amino acids was used, where the concentration of each amino acid was 50 or 100 μM), the GFP synthesis suddenly terminated after 30 to 40 min, whereas with an increased amino acid concentration (900 μM), the duration of synthesis was extended. The results shown here were obtained with a different batch of IVTT, and therefore, this result can only be compared qualitatively with those presented in the main text.

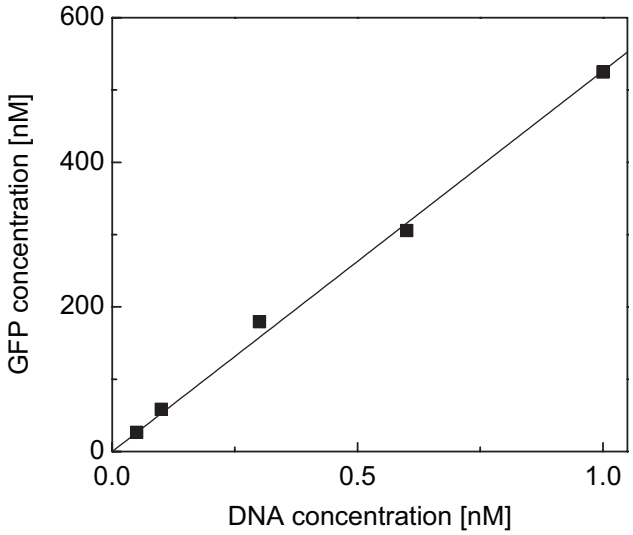

Figure S4: The relationship between the DNA concentration and the concentration of GFP after 2 h incubation at 37ºC in test tube. A linear relationship between the two can be seen. From the slope of this plot, we can obtain the number of GFP molecules synthesized per DNA molecule in a test tube, 530 GFP/DNA. In addition, as the efficiency of the GFP synthesis in the microchamber is 41% of that in the test tube, we can obtain the number of GFP molecules synthesized per DNA molecule in our microchamber, 220 GFP/DNA. If the protein synthesis proceeds until the substrates (amino acids or the ATP) run out, the concentration of the synthesized protein will become identical irrespective of the compartment size. However, the reaction terminates before substrate depletion because of the inactivation of the translation machinery. Therefore, the final GFP concentration and DNA concentration show a linear relationship.

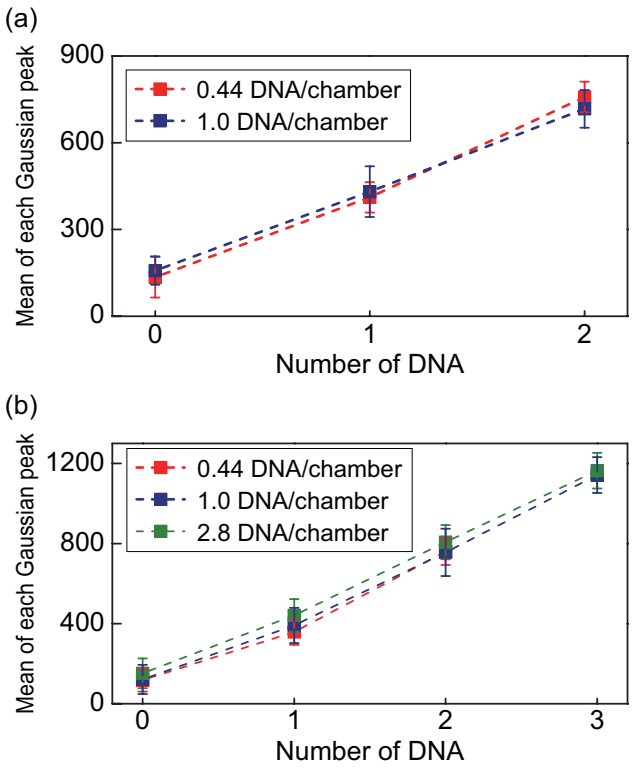

Figure S5: The relationship between the apparent DNA occupancy in each chamber and the mean fluorescence intensity obtained from fitting with the Gaussian function. The results with (a) GFP and (b) GAL are shown. The error bars indicate the standard deviation of each Gaussian function.

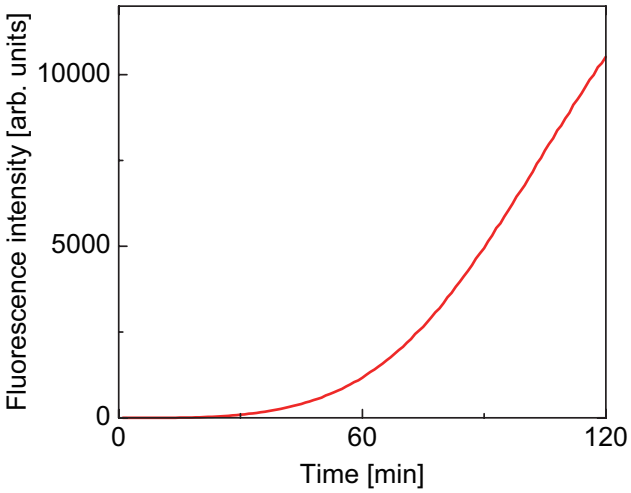

Figure S6: Time course of the GAL synthesis reaction in the test tube. DNA concentration was 76 p